hydrophobic interaction of pendent cholesteryl groups are effective in targeting polynucleotides immobilized on a nitrocellulose membrane (*Id.*). Fluorescein groups anchored in the membrane of the liposome were used as the reporter group. They served effectively, but sensitivity was limited by the fact that the signal from fluorescein in regions of high local concentration (*e.g.*, on the liposome surface) is weakened by self quenching.

The liposomes are made by methods well known in the art. See Zhang et al., *Tetrahedron Lett.*, **37**, 6243 (1996). The liposomes will generally be about 5-50 times larger in size (diameter) than the nanoparticles used in subsequent steps. For instance, for nanoparticles about 13 nm in diameter, liposomes about 100 nm in diameter are preferably used.

The liposomes bound to the substrate are contacted with a first type of nanoparticles having at least a first type of oligonucleotides attached thereto. The first type of oligonucleotides have a hydrophobic group attached to the end not attached to the nanoparticles, and the contacting takes place under conditions effective to allow attachment of the oligonucleotides on the nanoparticles to the liposomes as a result of hydrophobic interactions. A detectable change may be observable at this point.

The method may further comprise contacting the first type of nanoparticle-oligonucleotide conjugates bound to the liposomes with a second type of nanoparticles having oligonucleotides attached thereto. The first type of nanoparticles have a second type of oligonucleotides attached thereto which have a sequence complementary to at least a portion of the sequence of the oligonucleotides on the second type of nanoparticles, and the oligonucleotides on the second type of nanoparticles have a sequence complementary to at least a portion of the sequence of the second type of oligonucleotides on the first type of nanoparticles. The contacting takes place under conditions effective to allow hybridization of the oligonucleotides on the first and second types of nanoparticles. This hybridization will generally be performed at mild temperatures (e.g., 5°C to 60°C), so conditions (*e.g.*, 0.3-1.0 M NaCl) conducive to hybridization at room temperature are employed. Following

hybridization, unbound nanoparticle-oligonucleotide conjugates are washed from the substrate.

The combination of hybridizations produces a detectable change. The detectable changes are the same as those described above, except that the multiple hybridizations result in an amplification of the detectable change. In particular, since each of the liposomes has multiple oligonucleotides (having the same or different sequences) attached to it, each of the liposomes can hybridize to a plurality of the first type of nanoparticle-oligonucleotide conjugates. Similarly, since each of the first type of nanoparticles has multiple oligonucleotides attached to it, each of the first type of nanoparticle-oligonucleotide conjugates can hybridize to a plurality of the second type of nanoparticle-oligonucleotide conjugates. Also, the liposomes may be hybridized to more than one portion of the nucleic acid to be detected. The amplification provided by the multiple hybridizations may make the change detectable for the first time or may increase the magnitude of the detectable change. This amplification increases the sensitivity of the assay, allowing for detection of small amounts of nucleic acid.

If desired, additional layers of nanoparticles can be built up by successive additions of the first and second types of nanoparticle-oligonucleotide conjugates. In this way, the number of nanoparticles immobilized per molecule of target nucleic acid can be further increased with a corresponding increase in the intensity of the signal.

Also, instead of using second and third types of nanoparticle-oligonucleotide conjugates designed to hybridize to each other directly, nanoparticles bearing oligonucleotides that would serve to bring the nanoparticles together as a consequence of hybridization with binding oligonucleotides could be used.

Methods of making the nanoparticles and the oligonucleotides and of attaching the oligonucleotides to the nanoparticles are described above. A mixture of oligonucleotides functionalized at one end for binding to the nanoparticles and with or without a hydrophobic group at the other end can be used on the first type of nanoparticles. The relative ratio of these oligonucleotides bound to the average nanoparticle will be controlled by the ratio of

the concentrations of the two oligonucleotides in the mixture. The hybridization conditions are well known in the art and can be readily optimized for the particular system employed (see above).

An example of this method of detecting nucleic acid is illustrated in Figure 18. The hybridization of the first type of nanoparticle-oligonucleotide conjugates to the liposomes may produce a detectable change. In the case of gold nanoparticles, a pink/red color may be observed or a purple/blue color may be observed if the nanoparticles are close enough together. The hybridization of the second type of nanoparticle-oligonucleotide conjugates to the first type of nanoparticle-oligonucleotide conjugates will produce a detectable change. In the case of gold nanoparticles, a purple/blue color will be observed. All of these color changes may be observed with the naked eye.

In yet other embodiments utilizing a substrate, an "aggregate probe" can be used. The aggregate probe can be prepared by allowing two types of nanoparticles having complementary oligonucleotides (a and a') attached to them to hybridize to form a core (illustrated in Figure 28A). Since each type of nanoparticle has a plurality of oligonucleotides attached to it, each type of nanoparticles will hybridize to a plurality of the other type of nanoparticles. Thus, the core is an aggregate containing numerous nanoparticles of both types. The core is then capped with a third type of nanoparticles having at least two types of oligonucleotides attached to them. The first type of oligonucleotides has a sequence b which is complementary to the sequence b' of a portion of a nucleic acid to be detected. The second type of oligonucleotides has sequence a or a' so that the third type of nanoparticles will hybridize to nanoparticles on the exterior of the core. The aggregate probe can also be prepared by utilizing two types of nanoparticles (see Figure 28B). Each type of nanoparticles has at least two types of oligonucleotides attached to them. The first type of oligonucleotides present on each of the two types of nanoparticles has sequence b which is complementary to the sequence b' of a portion of the nucleic acid to be detected. The second type of oligonucleotides on the first type of nanoparticles has a sequence a which is complementary to the sequence a' of the second type of oligonucleotides on the second type